UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF FERMENTATION TECHNIQUE FOR HIGH CELL DENSITY CULTIVATION OF BAKER'S YEAST (SACCHAROMYCES CEREVISAE)

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IB 2005 8

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

April 2005

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DEVELOPMENT OF FERMENTATION TECHNIQUE FOR HIGH CELL DENSITY CULTIVATION OF BAKER'S YEAST (*SACCHAROMYCES CEREVISAE*)

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Chairman : Rosfarizan Mohamad, PhD

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The yeast, *Saccharomyces cerevisae*, isolated from fermented food was used in this study. Batch fermentation studies of *S. cerevisae* in a 2 L stirred tank fermenter were undertaken to generate kinetic growth data for the design of continuous and fed-batch fermentation. The variables studied in batch fermentation include the use of different concentrations of carbon and nitrogen sources and the effect of agitation speed (ranging from 200 to 1200 rpm), on the performance of each fermentation. The fermenter equipped with the multifermenter control system (MFCS) was used in exponential fed-batch fermentation to control the feeding rate of the glucose to the culture according to the proposed algorithm.

In batch fermentation, final cell concentration obtained increased proportionally with initial glucose concentration up to 120 g/L, which gave a constant cell yield of 0.13 g cell/g glucose. However, the specific growth rate (μ) reduced with increase in glucose

concentration. The amount of ethanol accumulated in the culture also increased proportionally with increasing glucose concentration. In term of overall productivity, the highest (0.35 g/L.h) was obtained in fermentation using 80-120 g/L glucose. Different growth characteristics of yeast were also observed at different agitation speeds.The final cell concentration increased from 29.76 g/L at agitation speed of 200 rpm to 41.90 g/L at 1000 rpm. However, a slight decrease in cell viability was observed with increasing agitation speed. The fermentation with controlled DOT throughout the fermentation (via agitation speed) did not improve the fermentation performance. For example, maximum cell concentration obtained in fermentation where DOT was controlled at 40% saturation was only 20.38 g/L.

From this study, it can be suggested that the optimal medium composition and culture condition for batch cultivation of Baker's yeast are as follows; glucose (100 g/L); yeast extract (25.0 g/L); peptone (11.80 g/L); agitation speed (1000 rpm); air flow rate (1 vvm); DOT not controlled; and pH controlled at 5.5. In this fermentation run, the final cell concentration obtained was 41.90 g/L which gave the cell yield and overall productivity of 0.24 g/g and 2.41 g/L.h, respectively. Although higher overall productivity was obtained in continuous culture (5.53 g/L.h) operated at a dilution rate of 0.3 h^{-1}, the concentration of cell (18.43 g/L) in outflow was very much lower than in batch culture. In addition, the cell yield obtained in continuous culture 0.21 g/g was slightly lower than those obtained in batch fermentation.

The models based on Monod and Luedeking-Piret equations were found suitable to describe the growth of *S. cerevisiae*, glucose consumption and ethanol production in

batch and continuous fermentation processes. Kinetic parameters such as μ_{max}, K_s and $Y_{x/s}$ were estimated and used to verify the experimental data.

High cell density cultivation was achieved in exponential fed-batch fermentation with the feed rate of the substrate increased according to the exponential growth of the yeast at specific growth rate (0.1 to 0.4 h^{-1}) below the maximum. The highest cell concentration (89.97 g/L) was obtained at specific growth rate of 0.1 h^{-1}, which was associated with very small quantity of ethanol accumulated and residual sugar was not detected in the culture during the fermentation. The cell yield (0.72 g cell/g glucose) and overall productivity (3.8 g/L.h) obtained in fed-batch fermentation was significantly higher than those obtained in batch fermentation. The sugar limitation that was maintained during exponential fed-batch fermentation was successfully utilized to enhanced biomass yield and substrate cultivation hence.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBANGUNAN TEKNIK FERMENTASI UNTUK MENGHASILKAN SEL YIS ROTI BERKETUMPATAN TINGGI (*SACCHAROMYCES CEREVISAE*)

Oleh

AHMAD ARIFF

April 2005

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Yis, *Saccharomyces cerevisiae,* yang telah dipencilkan daripada makanan tertapai digunakan dalam kajian ini. Kajian fermentasi sesekelompok keatas pengkulturan *S. cerevisiae* menggunakan fermenter berpengaduk mekanikal 2 liter telah dijalankan untuk menghasilkan data pertumbuhan kinetik bagi yis. Data ini seterusnya digunakan sebagai maklumat asas untuk merancang dan mereka bentuk kaedah fermentasi selanjar dan suapan sesekelompok eksponen bagi meningkatkan prestasi pengkulturan yis tersebut. Pembolehubah dan parameter yang dikaji dalam fermentasi sekelompok termasuklah penggunaan kepekatan sumber karbon dan nitrogen yang berbeza, dan juga kesan kelajuan pengaduk (julat daripada 200 kepada 1200 rpm) keatas prestasi pengkulturan. Sistem Kawalan Beberapa Fermenter (SKBB) telah digunakan dalam operasi fermenter suapan sesekelompok untuk mengawal kadar suapan glukosa ke dalam kultur mengikut algrotihma yang telah dicadangkan.

Kepekatan sel akhir didapati meningkat dengan kepekatan glukosa yang dibekalkan sehingga kepada kepekatan 120 g/L yang telah memberikan nilai angkali hasil sel yang hampir sama pada 0.13 g sel/g glukosa. Walaubagaimanapun, kadar pertumbuhan spesifik yis berkurangan dengan pertambahan kepekatan glukosa. Amaun etanol yang terkumpul di dalam kultur juga meningkat bersepadanan dengan pertambahan kepekatan glukosa. Produktiviti keseluruhan yang tertinggi (0.35 g/L. j) telah dicapai dalam fermentasi yang menggunakan 80 – 120 g/L glukosa. Ciri-ciri pertumbuhan yis yang berbeza juga diperhatikan pada kelajuan pengaduk yang berbeza, yang telah membekalkan kadar pemindahan isipadu oksigen (K_La) yang berbeza, kepada kultur. Walaubagaimanapun, sedikit pengurangan ke atas peratus sel yang hidup dengan peningkatan kelajuan pengaduk. Fermentasi dengan kawalan ketegangan oksigen terlarut (KOT) (melalui kelajuan pengaduk) tidak meningkatkan prestasi proses fermentasi. Contohnya, kepekatan sel maksimum yang di dapati dalam fermentasi kawalan KOT pada 40%, hanya 20.38 g/L.

Daripada kajian yang telah dijalankan, dapat dicadangkan kandungan medium dan keadaan kultur optima untuk pertumbuhan yis roti adalah seperti berikut; glukosa (100 g/L); ekstrak yis (25.0 g/L); pepton (11.8 g/L); kelajuan pengaduk (1000 rpm); kadar pengaliran udara (1 vvm); KOT tidak perlu di kawal; dan pH di kawal pada 5.5. Dalam proses fermentasi yang telah dijalankan, didapati kepekatan sel akhir ialah 41.90 g/L, hasil sel dan produktiviti keseluruhan masing-masing ialah 0.24 g/g dan 2.41 g/L.j. Walaupun nilai produktiviti keseluruhan (5.53 g/L.j) yang tinggi diperoleh melalui proses fermentasi selanjar, tetapi kepekatan sel (18.83 g/L) dalam aliran keluar fermenter adalah lebih rendah daripada kultur sekelompok dan perkara ini tidak menarik

kerana akan meningkatkan kos pemulihan hasil. Tambahan pula, dalam fermentasi selanjar hasil sel yang didapati ialah 0.21 g/g adalah lebih rendah berbanding dalam fermentasi sesekelompok.

Model menggunakan persamaan Monod dan Luedeking-Piret telah didapati sesuai untuk menerangkan pertumbuhan *S. cerevisiae* dengan menggunakan glukosa, iaitu dalam proses fermentasi sesekelompok dan selanjar. Setiap nilai parameter kinetik seperti μ_{max}, K_s and $Y_{x/s}$ telah dianggarkan dan dibandingkan.

Pengkulturan sel pada kepadatan yang tinggi telah dicapai menggunakan kaedah fermentasi suapan sesekelompok, dengan kadar suapan substrat meningkat bersamaan dengan pertumbuhan eksponen yis pada kadar pertumbuhan spesifik di bawah nilai maksimum. Pada julat kadar pertumbuhan spesifik (0.1 kepada 0.4 j^{-1}) yang digunakan dalam operasi fermentasi suapan sesekelompok eksponen, kepekatan sel tertinggi (89.97 g/L) telah dicapai pada kadar pertumbuhan spesifik 0.1 j^{-1}. Dalam proses fermentasi ini, amaun etanol yang terkumpul adalah sangat rendah dan tiada sisa glukosa yang dikesan dalam kultur sepanjang proses fermentasi. Nilai angkali hasil sel (0.72 g sel/g glukosa) dan produktiviti keseluruhan (3.8 g/L.j) yang dicapai dalam fermentasi suapan sesekelompok, adalah lebih tinggi berbanding yang diperoleh dalam fermentasi sesekelompok. Pengawalan kepekatan glukosa pada aras yang rendah dalam kultur yang boleh dicapai dalam proses fermentasi suapan sesekelompok telah berjaya meningkatkan penghasilan biojisim.

ACKNOWLEGDEMENTS

Firstly, I would like to thank to my supervisor, Dr. Rosfarizan Mohamad for accepting me, without any biotechnology background, as her post-graduate student. Without the opportunity and commitment given, my part time study on the Fermentation Technology area might not be possible. I also would like to express my appreciation, from the bottom of my heart, to members of the supervisory committee, Assoc. Prof. Dr. Raha Abdul Karim and Prof Dr. Mohamed Ismail Abdul Karim, for their invaluable guidance, never ending patient, kind assistance and time. Their fruitful suggestions and comments on technical and theoritical have been of great value. Special thanks and appreacition also goes to Dr Hirzun Mohd Yusoff who at first accepted me as his student, who then moved to Sime Darby Sdn. Bhd. after second semester of my study. Finally, I wish to thank to all postgraduate students and laboratory staff, of Fermentation Technology Unit, Enzyme and Microbial Technology Laboratory, Institute of Bioscience for their assistance and favor throughout of the length of my study.

I certify that an Examination Committee met on 27th April 2005 to conduct the final examination of Ahmad Bin Ariff on his Master of Science thesis entitled "Development of Fermentation Technique for High Cell Density Cultivation of Baker's Yeast" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science.The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work expect for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at Universiti Putra Malaysia or other institutions.

AHMAD ARIFF

Date: 18 JUL 2005

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LIST OF ABBREVIATIONS

C/N	Carbon to nitrogen ratio of medium in mM basis
D_i	Impeller diameter
DOT	Dissolved oxygen tension
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate of yeast
m	Growth associated rate constant for Baker's yeast production (g product/g cell)
n	Non-growth-associated constant for Baker's yeast production (g product/g cell.h)
S_i	Initial substrate concentration (g/L)
T	Fermentation time (h)
X	Cell concentration (g/L)
X_i	Initial cell concentration (g/L)
X_{max}	Maximum cell concentration (g/L)
$Y_{x/s}$	Yield of cell on the basis of substrate consumed (g cell/g substrate)

CHAPTER 1

INTRODUCTION

The word 'yeast' is not easily defined, but basically yeasts are recognized as being unicellular fungi. More definitively: 'Yeasts are ascomycetous or basidiomycetous fungi that reproduce vegetatively by budding or fission, and that form sexual states which are not enclosed in a fruiting body' (Boekhout and Kurtzman, 1996). Except when we refer to other species of yeast by name, we will use the term "yeast" to refer to *S. cerevisiae*. In order to grow, yeast needs suitable sources of carbon, energy, nitrogen and phosphorus. Other essential compounds include potassium, magnesium, calcium and iron. Certain trace elements and growth factor must also be present in the medium has to be strongly aerated, oxygen present in air being a necessary raw material in yeast cultures. In addition, the process requires acid for pH-regulation, antifoams agents, and water to make the medium and for cooling. Yeast have a simple nutritional needs. Unable to carry out photosynthesis, they require a reduced carbon source which can be as simple a compound as acetate. In addition, they also require nitrogen source such as ammonium sulphate. Yeasts can use a variety of organic nitrogen compounds, including urea and various amino acids. The only other complex compound that they require is the vitamin, biotin.

Ascomycetes, such as Baker's yeast, are popular for genetics research because the ascospores they produce in each ascus are the products of meiosis. When yeast are nutritionally stressed, for example by deprivation of either a carbon source or a nitrogen source, diploid yeast will sporulate. The ease with which Baker's yeast can

be maintained as either haploid cells or diploid cells is another characteristic that makes them attractive to geneticists. Other genera of yeast also have practical uses. Some can use hydrocarbons, such as petroleum, as a carbon source. These organisms can literally convert petroleum into protein. They are being used to remove petroleum as a pollutant from the environment and to convert low-grade hydrocarbons into protein for consumption by animals.

Yeast metabolism uses nutrients to synthesis new cell material as well as to generate energy for survival. Metabolically, yeast are mostly facultative aerobes, capable of growing either in the absence of air (fermentative) or in its presence (oxidative). Therefore, in the absence of aeration, yeast has ability to instantaneously change its respiratory metabolism from oxidative to fermentative. This catabolic shift is reffered to as the 'Pasteur effect" or "Glucose effect". Under aerobic growth conditions they can support growth by oxidizing simple carbon sources, such as ethanol, acetate or glycerol. If they have adequate oxygen, they will completely oxidize carbon sources, usually sugars, to carbon dioxide and water. However, under anaerobic conditions, yeast can convert sugars only to carbon dioxide and ethanol, recovering less of the energy. In either case, growth will be limited by some essential nutrient or the accumulation of the toxin.

Yeast has major economic, social and health significance in human health. Yeast (as natural strain and host cell for recombinant strain) are one of the most extensively utilized microorganisms in industrial biotechnology. Baker's yeast are used in the production of fermented food products such as alcohol and breads. Ethanol is a valuable alternative to petroleum as raw material for the manufacturing of many

important commercial chemicals. In particular, genetically manipulated yeasts can now be exploited to produce many different biopharmaceutical agents for preventing and reating human diease. Due to dwindling availability of fossil fuel, microbial production of bio-fuel from organic by products has acquired significant importance in recent years. Ethanol has been identitied as an alternative fuel for the future. Even though several microorganisms, including *Clostridium* sp., have been considered as ethanologenic microbes, the yeast *S. cerevisiae* and facultative bacterium *Zymomonas mobilis* are better candidates for industrial alcohol production. Some biological processes have rendered possible routes for producing ethanol and methane in large volumes. A worldwide interest in the utilization of bio-ethanol as an energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production. Linden et al. (1985) recognised ethanol has a commercial application in chemical and cosmetic industry. According to Kolot (1984), ethanol can be used as a solvent for oil and fats processing in chemical industry and also used as fuel (Kolot, 1984). Bio-ethanol is preferred over synthetic ethanol for applications involving human consumption such as in pharmaceuticals and toiletries. Ethanol from fermentation process normally commands a premium price over synthetic ethanol.

In future, yeast exploitation is likely to make significant impact in relation to renewable energy supply, environmental biotechnology including biological control and in health-care issues, particularly the study of human genetic disorders and cancer. People have used yeast, for controlled fermentation of food and drink and for leavening in baking throughout recorded history. In fact, the brewing of beer probably represented the world's first biotechnology. Today, there are also used in a

variety of commercial fermentation and biomass conversion process. Their usefulness is based on their ability to convert sugars and other carbon sources into ethanol in the absence of air (anaerobic), and into carbon dioxide and water in the presence of air (aerobic). Yeast as food, is rich in protein and is an uncommonly good source of the B vitamins. It provides a valuable source of nutrients that are important in low-meat or vegetarian diets.

The growth characteristics of *S. cerevisae* are varied depending on the culture conditions and medium composition. Growth of yeast is greatly influenced by the level of dissolved oxygen concentration in the culture and the different carbon sources used. The growth kinetics of yeast in adaptation to variation in sugar concentration has also been well documented.

In order to develop industrial production of yeast biomass, the process must be economic and efficient in term of substrate utilization (i.e high cell yield), high final cell concentration in fermenter (i.e., to reduce recovery cost and size of fermenter), and high overall productivity. This study is aimed at the development of an efficient cultivation technique for the production of locally isolated Baker's yeast *S. cerevisiae*. Thus, the objectives of the study are;

1) To identify and characterize the locally isolated Baker's yeast from food product which has potential for commercialization.
2) To study the effect of different medium formulations and culture conditions on the growth characteristics of *S. cerevisiae*.